REMARKS

The above amendments to the above-captioned application along with the following remarks are being submitted as a full and complete response to the Official Action dated October 1, 2003. In view of the above amendments and the following remarks, the Examiner is respectfully requested to give due reconsideration to this application, to indicate the allowability of the claims, and to pass this case to issue.

Status of the Claims

Claims 2 and 4 are under consideration in this application. Claim 2 is being amended, as set forth in the above marked-up presentation of the claim amendments, in order to more particularly define and distinctly claim applicants' invention.

Additional Amendments

Claim 2 is being amended to correct formal errors and/or to better recite or describe the features of the present invention as claimed. All the amendments to the claims are supported by the specification. Applicants hereby submit that no new matter is being introduced into the application through the submission of this response.

Prior Art Rejections

Claims 2 and 4 were rejected under 35 U.S.C. § 102(b) on the grounds of being anticipated by an article by Schollien et al. published on Clinical Chemistry, Vol. 43, 1997, No.1, pp. 18-23 (hereinafter "Schollien"). Claims 2 and 4 were further rejected under 35 U.S.C. § 103(a) on the grounds of being unpatentable over an article by Pastinen et al. published on Genome Research, Vol. 7(6), 1997, pp. 606-614 (hereinafter "Pastinen") in view of U.S. Pat. No. 6,589,778 to Hawkins (hereinafter "Hawkins"); and Pastinen in view of U.S. Pat. No. 5,232,829 to Longiaru et al. (hereinafter "Longiaru") and Hawkins. These rejections have been carefully considered, but are most respectfully traversed.

The method of detecting PCR-amplified base sequences 42 of the invention, as now recited in claim 2, comprises: conducting PCR amplification by mixing a plurality of primer pairs with a sample, said primer pairs being suitable for amplifying different base sequences, such as DNA (1), DNA(2), of a same length or different lengths by PCR; **conducting a hybridization reaction by using a substrate 30 on which <u>one primer of each of said primer pairs</u> used for**

the PCR (e.g., primers 31, 32, 33 which are three types of forward primers of the same length, page 9, lines 7, 12-26, Fig. 3), **are fixedly spotted on spots thereon and a solution containing said base sequences that are PCR-amplified in the preceding step**, said hybridization reaction being performed between the primers fixedly spotted on the substrate and said PCR-amplified base sequences; and detecting at least one of the spots on said substrate in which the hybridization reaction occurs. Different types of primers 31, 32, 33 (which may be of the same length) are fixed/implanted on different spots of a glass slide/ substrate 30 (page 9, lines 15-18) to hybridize with different types of DNAs so as to measure the fluorescence emitted from each spot corresponding to one type of PCR-amplified DNAs 42 derived from a respective type of primer (page 10, lines 25-28) and thereby determine the amount of different types of PCR-amplified DNAs. The spot detecting step involves **processing an intercalating dye 46** ("*a reagent specifically reactive with double-stranded DNA, such as ethidium bromide, cyber green, Hoechst 33258 or the like, for the purpose of dyeing thereof*" page 10, line 4-5) **to enter in said PCR-amplified base sequences 42 which are double-stranded DNAs**(page 10, lines 10-28; Fig. 4); **and detecting fluorescence generated by exciting said intercalating dye contained in said at least one of the spots on the substrate**. In particular, the invention performs hybridization on the primers used for PCR amplification, and then intercalating dye is entered into PCR-amplified double-stranded DNAs (hybridization products).

By using a hybridization probe having the same sequence as the primer used for PCR, there is no need to independently/ separately develop a hybridization probe. As a result, design time and manufacture cost are significantly reduced. It often take a lot of time to design a primer for detecting RNA-type viruses, especially those having many mutations (a designed primer is not necessarily capable of performing PCR amplification or hybridization).

The application of intercalating dye was derived from the use of the PCR primer as a hybridization probe. Applying any fluorescent-labeled probe would defeat the purpose of the invention eliminate separately synthesized fluorescent-labeled probes. For this reason, the invention abandoned the conventional fluorescent labeling but applying an intercalating dye, i.e., a post-dyeing method, so that detection can be made without providing fluorescent-labeling (or RI labeling) probes.

Using intercalating dyes has been known but not popular due to the fact that the post-dyeing technique (using intercalating dye) is not as sensitive as the technique using fluorescent-

labeled probes. However, in the present invention, reliable detection is made possible by carrying out hybridization by the PCR method using the PCR primers as hybridization probes then entering intercalating dye in PCR-amplified double-stranded DNAs (hybridization products).

Applicants respectfully contend that none of the cited reference teach or suggest "performing hybridization on the primers used for PCR amplification" then "entering intercalating dye in PCR-amplified double-stranded DNAs (hybridization products)".

In contrast, in Schollien, the sequences of the primers used for PCR amplification as described in "Table 1. Primers for LHON multiplex PCR" (page 19) are different from the sequences of the oligonucleotides for hybridization as described in "Table 2. Oligonucleotides used in the RDB assay" (page 20). In other words, in Schollien, hybridization is performed using oligonuclcotide sequences different from the primers used for PCR amplification. In addition, as admitted by the Examiner (page 3, 1st paragraph; page 5, 5th to 4th lines from the bottom of the outstanding Office Action), "*Schollien did not teach an intercalating agent as a fluorescent material*". Much less does Schollien teach "entering intercalating dye in PCR-amplified double-stranded DNAs (hybridization products)".

Pastinen shares the same deficiencies as Schollien. As admitted by the Examiner (page 7, 3rd paragraph of the outstanding Office Action), "*Pastinen did not specifically teach said PCR primers used as probes in hybridization reaction.* " In addition,

The sequences of the PCR primers are different from the sequences of the Minisequencing primer as described in "Table 2. Genes, Primers, and Mutations" on page 612 of Pastinen. Thus, Pastinen fails to perform hybridization on the primers used for PCR amplification. In addition, Pastinen fails to teach "using an intercalating agent as a fluorescent material" or "entering intercalating dye in PCR-amplified double-stranded DNAs (hybridization products)".

Hawkins was relied upon by the Examiner to teach intercalating dyes. Hawkins fails to compensate for Schollien's and Pastinen's deficiencies since it merely discusses intercalating dyes generally, but fails to disclose "performing hybridization on the primers used for PCR amplification" or "entering intercalating dye in PCR-amplified double-stranded DNAs (hybridization products)" of the invention.

Longiaru was relied upon by the Examiner to teach using PCR primers as capture probes in hybridization reaction. However, Longiaru actually teach away from the invention by using

PCR primers as capture probes for capturing labeled amplicon in wells on a microtiter plate ("*the present invention utilizes fixation of a capture DNA sequence to microtiter wells and subsequent detection by hybridization to* ***labeled*** *(viz. biotinylated) amplicons*" col. 1, lines 60-63; col. 7, line 11). On the other hand, the invention uses probes immobilized on a substrate to detect ***unlabelled*** PCR-amplified base sequences. As mentioned, the invention uses the same sequences, i.e., chemically identical, as the primers for PCR amplification and the probes for hybridization which excludes detection methods based on fluorescent-labeled DNA, radio-active substance labeled DNA or chiemilumincescence, and chemically identical DNA probes such that the invention requires applying intercalating dyes for detection. It is well established that a rejection based on cited references having principles that teach away from the invention is improper.

By analogy, one skilled in the art will not be motivated to combine the teachings for capturing labeled amplicons in Longiaru and the teachings of intercalating dyes in Hawkins in the manner suggested by the Examiner since intercalating dyes exclude the need to label the amplicons. It is well established that a rejection based on combining cited references having contradictory principles is improper.

Although the invention applies the general mechanism of intercalating dye as disclosed in Hawkins, the invention enters the intercalating dye in PCR-amplified double-stranded DNAs (hybridization products) to achieve the above-mentioned unexpected results or properties, such as eliminating separately synthesized fluorescent-labeled probes, reliable detection achieved by entering intercalating dye in PCR-amplified double-stranded DNAs. The presence of these unexpected properties is evidence of nonobviousness. MPEP§716.02(a).

> "*Presence of a property not possessed by the prior art is evidence of nonobviousness. In re Papesch, 315 F.2d 381, 137 USPQ 43 (CCPA 1963) (rejection of claims to compound structurally similar to the prior art compound was reversed because claimed compound unexpectedly possessed anti-inflammatory properties not possessed by the prior art compound); Ex parte Thumm, 132 USPQ 66 (Bd. App. 1961) (Appellant showed that the claimed range of ethylene diamine was effective for the purpose of producing " 'regenerated cellulose consisting substantially entirely of skin' " whereas the prior art warned "this compound has 'practically no effect.' ").*

The unexpected properties were unknown and non-inherent functions in view of

Hawkins, since Hawkins does not inherently achieve the same results. In other words, these advantages would not flow naturally from following the teachings of Hawkins, since Hawkins fails to suggest "entering the intercalating dye in PCR-amplified double-stranded DNAs (hybridization products)".

Applicants further contend that the mere fact that one of skill in the art could accidentally enter Hawkins's intercalating dye into PCR-amplified double-stranded DNAs (hybridization products) to meet the terms of the claims is not by itself sufficient to support a finding of obviousness. The prior art must provide a motivation or reason for one skilled in the art to provide the unexpected properties, such as eliminating separately synthesized fluorescent-labeled probes, or reliable detection, without the benefit of appellant's specification, to make the necessary changes in the reference device. *Ex parte Chicago Rawhide Mfg. Co.*, 223 USPQ 351, 353 (Bd. Pat. App. & Inter. 1984). MPEP§2144.04 VI C.

As such, the present invention as now claimed is distinguishable and thereby allowable over the rejection raised in the Office Action. The withdrawal of the outstanding prior art rejections is in order, and is respectfully solicited.

In view of all the above, clear and distinct differences as discussed exist between the present invention as now claimed and the prior art reference upon which the rejections in the Office Action rely, Applicants respectfully contend that the prior art references cannot anticipate the present invention or render the present invention obvious. Rather, the present invention as a whole is distinguishable, and thereby allowable over the prior art.

Favorable reconsideration of this application is respectfully solicited. Should there be any outstanding issues requiring discussion that would further the prosecution and allowance of

the above-captioned application, the Examiner is invited to contact the Applicants' undersigned representative at the address and phone number indicated below.

Respectfully submitted,

Stanley P. Fisher
Registration Number 24,344

Juan Carlos A. Marquez
Registration Number 34,072

REED SMITH LLP
3110 Fairview Park Drive, Suite 1400
Falls Church, Virginia 22042
(703) 641-4200

December 22, 2003

SPF/JCM/JT